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Perceived Stress is associated with Accelerated Monocyte/ Macrophage Aging Trajectories in Clinically Normal Adults

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Abstract

Objectives—Chronic stress is associated with poorer age-related cognition, but the mechanisms of this relationship are not well understood. Aging increases expression of activated macrophages, leading to exacerbated immune responses to stressors. We examined the impact of stress and aging on macrophage-related inflammation and cognition in clinically normal adults.

Methods—380 clinically normal adults were followed longitudinally (age M=73y; visit range 1–8, M=2.5 visits). Participants completed the Perceived Stress Scale, a neuropsychological battery, and blood draws. Plasma was analyzed for cytokines related to macrophage function: IL-6, TNF-α, MIP1-α, MIP1-β. Linear mixed-effects examined the effects of age, baseline stress, and their interaction predicting macrophage cytokines, adjusting for sex, education, and depressive symptoms. Latent growth curve models assessed the mediating role of macrophage cytokines on the relationship between age and cognition in high or low stress.

Results—Baseline perceived stress interacted with age to predict macrophage cytokines longitudinally. Specifically, high stress adults demonstrated accelerated age-related elevations in macrophage cytokines across time. Macrophage cytokines negatively tracked with executive functioning longitudinally. Macrophage cytokines mediated 19% of the relationship between age and executive functions in high, but not low stress adults.

Conclusions—Our data provide evidence of accelerated immune aging among individuals with high stress. Elevated macrophage cytokine trajectories mediated the effect of age on executive functions only in individuals with high stress suggesting these constructs may be more tightly linked in elevated stress contexts. Stress interventions are warranted to optimize the immune aging with possible downstream cognitive benefits even among clinically normal adults.

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Keywords

Inflammation; Macrophage inflammatory proteins; Interleukin-6; Tumor necrosis factor-alpha; Episodic memory

INTRODUCTION

Chronic stress is associated with poorer brain health and less successful cognitive aging[1,2]. Peripheral cortisol (a stress hormone indicating elevated stress exposure) and reported stress levels are consistently associated with worse cognitive performances, and smaller hippocampal and prefrontal cortical volumes among older adults cross-sectionally and predict steeper rates of cognitive decline longitudinally (e.g., 7- and 20-year study periods)[1–5]. The Vietnam Era Twin Study of Aging further demonstrated that the robust deleterious impact of chronic stress (as measured with cortisol) on cognition and cortical volumes were independent of premorbid or genetic factors, underscoring the importance of adverse *environmental* experiences on cognitive aging[6]. Despite the consistency of this relationship, the underlying mechanisms of how chronic stress may potentiate cognitive aging in humans are not well understood.

Interestingly, both repetitive stress exposure and aging independently disrupt neuroinflammatory pathways, and together, may produce an exaggerated state of chronic inflammation. In normal aging, a collective state of immunosenescence occurs in which remodeling of both the adaptive and innate immune systems results in increased susceptibility to infectious diseases, neoplasms, and other autoimmune conditions[7,8]. Importantly, these disrupted immune responses are detrimentally associated with indicators of neural health (e.g., fMRI connectivity, cortical atrophy, cognitive impairment) and are causally implicated in the pathological development of neurodegenerative disorders, such as Alzheimer's disease[9–11]. Chronic exposure to both psychological and peripheral physical stressors trigger immune responses that are associated with disrupted brain functioning[12,13]. Drawing on animal models of CNS functioning, while acute stressors result in brief, adaptive neuroinflammatory cascades that support learning and memory, recurrent exposure to stressors results in a prolonged pro-inflammatory neural state that inhibits long-term potentiation, promotes protein misfolding, and decreases neurotoxin clearance[4,14]. In clinical studies, negative emotions and stressful life experiences are associated with greater risk of and prolonged infection, delayed wound healing, and sustained pro-inflammatory cytokine production[15,16]. For example, adult caregivers demonstrated significantly increased production of IL-6 than their non-caregiving peers across a six-year period, regardless of whether they were actively caregiving over the study period[17]. Notably, these relationships may function in a bidirectional feedback loop such that greater inflammation may also lead to heightened perceived stress, anxiety, and mood symptoms[18]. Therefore, in the context of aging, chronic stressors may represent a "double hit" to an already vulnerable aging immune system resulting in greater inflammatory dysregulation[19] and poorer clinical outcomes.

The effects of age-related immunosenescence are postulated to affect immune cells involved in both innate and secondary pathogen responses, including reduced neutrophil activation,

fewer antigen-naïve and immature lymphocytes, as well as both under- and over-activation of monocytes[20]. Regarding the latter, macrophages have been posited to play a particularly relevant role in the aging-stress immune response. Macrophages are among the first line of immune defense mediating toxin and cell debris phagocytosis and signaling downstream cascades of cytokine and chemokine inflammatory regulating proteins[21]. Under normal conditions, macrophages rest in a quiescent state. However, during stress, disease, or infection, macrophages undergo morphological changes to become activated, releasing cytokines and launching a protective inflammatory response[22]. In aging, there appears to be a homeostatic shift such that not only the proportion of activated macrophages increases, but an additional subset of macrophages maintain an intermediate "primed" state[7,22]. In the "primed" state, macrophages are not appreciably secreting cytokines, but can be more rapidly induced, demonstrating greater cytokine release upon activation compared to nonprime macrophages. This disrupted macrophage signaling then has downstream effects on monocytic dendritic cells, which critically integrate communication between macrophages and lymphocytes (natural killer cells, and B and T cells) to mediate immune responses. For example, dendritic cells have demonstrated inappropriate persistence of pro-inflammatory cytokine production in response to pathogen exposure in older adults[23,24]. Therefore, exposure to chronic stress in the context of age-related elevations in basal inflammatory levels may result in exaggerated immune reactivity resulting in a prolonged "sickness response" (e.g., prolonged inflammation, decreased wound healing) in older adults.

In the current study, we aimed to determine the longitudinal impact of baseline perceived stress and aging on cytokine markers associated with monocyte/macrophage activation and cognition among neurologically normal older adults. We hypothesized that higher baseline perceived stress would potentiate age-related increases in monocyte/macrophage-related inflammation and cognitive decline. Identification of the neurobiological pathways by which stress impacts cognitive aging may be used to enhance intervention targets and therapy potencies. Given availability of empirically-supported behavioral therapies, stress may be a modifiable factor to promote successful cognitive aging.

METHODS

Participants

We recruited 380 community-dwelling older adults (baseline ages 55–99 years) followed as part of a larger, ongoing Healthy Cognitive Aging study at the University of California, San Francisco Memory and Aging Center. The UCSF Healthy Cognitive Aging study observationally follows 698 typically aging adults longitudinally with the aim of deeply characterizing the neurobiological and behavioral phenotypes of cognitive aging (see Figure 1 for attrition rates in current study). Participants complete prospective comprehensive evaluations at approximately 15-month intervals as part of the study, including questionnaires (e.g., Perceived Stress Scale), blood draws, and computerized neuropsychological assessment. Participants who completed the measures of interest (i.e., Perceived Stress Scale, cognitive testing, blood draws), were neurologically and neuropsychologically within normative standards per consensus research criteria[25], had the

able to independently complete activities of daily living operationalized by a 0 on the Clinical Dementia Rating scale via interviews with study partners, and had no major memory concerns or diagnosed memory condition at all study visits were selected for inclusion in the current analyses. Therefore, these data represent older adults who are neurologically normal at baseline and each follow-up visit (see Table 1).

Perceived Stress Scale (PSS)

The PSS is a 10-item measure assessing degree of life stress over the past month (e.g., "how unpredictable, uncontrollable, and overloaded respondents find their lives")[26]. Likert-scale answers range from "never," (0 points) to "very often" (4 points). Items are summed such that higher scores indicate greater levels of perceived negative stress. The PSS demonstrates good internal consistency[27] and strong ecological validity predicting health-related outcomes[17,26].

Laboratory measures of macrophage-related cytokines

We selected macrophage inflammatory protein 1-alpha (MIP1-α; CCL3), macrophage inflammatory protein 1-beta (MIP1-β; CCL4), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) *a priori* as our pro-inflammatory monocyte/macrophage-related cytokines of interest. Specifically, MIP1-α and MIP1-β were targeted due to their relatively more selective role in monocyte/macrophage inflammatory responses; these are chemotactic cytokines that are largely produced by monocytes (macrophages and dendritic cells), and are involved in both first-line innate immune activation as well as maintenance of immune homeostasis[28–30]. However, it is important to note that MIP1-α and MIP1-β are not exclusively produced by monocytes and in fact are additionally secreted by lymphocytes, endothelium, and fibroblast cells. Additionally, they target a host of different cell types including but not limited to monocytes, as well as migration of lymphocytes and granulocytes[31,32]. IL-6 and TNF-α were theoretically selected because they are both stimulated by and further potentiate MIP1-α and MIP1-β signaling[33,34]. Additionally, given that IL-6 and TNF-α are the primary cytokines that have been reported in the current human stress-inflammation literature, they were included to be comparable with existing studies[12,17]. Nonetheless, though importantly involved in monocyte/macrophage-mediated immune responses, IL-6 and TNF-α are again not entirely specific to this immune pathway and are pleotropic cytokines produced by and involved in the regulation of a host of cell types (e.g., endothelial cells, granulocytes, lymphocytes, adipocytes, heptocytes).[35–37]

Blood draws were completed at every study visit. Following an overnight 12-hour fast, blood was collected in the morning and centrifuged at 2000× g for 15 minutes at 4°C. The resultant plasma was transferred to 500uL polypropylene cryovials for long-term storage at −80°C until analysis. Prior to assay initiation, samples were gradually allowed to come to room temperature. Plasma measures of IL-6, TNF-α, MIP1-α, and MIP1-β were obtained using the Human Proinflammatory Panel 1 V-PLEX (IL-6, TNF-α) and Human Chemokine Panel 1 V-PLEX (MIP1α, MIP1β) kits provided by Meso Scale Diagnostics (Rockville, MD, USA). All assays were conducted according to manufacturer provided protocol. Each multiplex array was scanned using the Mesoscale QuickPlex SQ 120. Concentrations of IL-6, TNFα, MIP-α, and MIP1β were quantified using Discovery Workbench v4.0,

manufacturer provided software, based on sample dilution and relative to the supplied in-assay standard curve. Lower limit of detectability (LLOD), lower limit of quantification (LLOQ), and upper limit of quantification (ULOQ) were as follows for each marker (in pg/mL): IL-6: LLOD=0.01–0.11, LLOQ=1.58, ULOQ=488; TNF-α: LLOD=0.01–0.13, LLOQ=0.69; ULOQ=248; MIP1-α: LLOD=2.28–4.01, LLOQ=13.8, ULOQ=7.43; and MIP1-β LLOD 0.22–0.72, LLOQ=2.27, ULOQ=750. Sample measurements with a coefficient of variance (CV) greater than 20% across plates, or determined to be outliers with a value higher than three times the upper quartile range were excluded from subsequent analyses.

To capture a global index of macrophage-related inflammatory state, a composite score was calculated by converting raw cytokine values to continuous *z*-scores based on sample baseline values for each marker. The subsequent MIP1-α, MIP1-β, TNF-α, and IL-6 z-scores were then averaged together at each time point for an overall macrophage composite score.

Neuropsychological Battery

Episodic Memory—To measure verbal memory, we administered the California Verbal Learning Test-second edition (CVLT-II)[38]. Participants were asked to recall a 16-item word list that can be grouped into semantic categories across five learning trials. Participants were then asked to freely recall the list after an interference trial, and again after a 20–30 minute delay. Our primary memory outcome on the CVLT-II was free recall after 20–30 minutes (possible range 0–16 words).

Executive Functioning—To assess executive functioning, a composite score was derived from five computer-based tests of working memory (Dot Counting, 1-Back, 2-Back), response inhibition (Enclosed Flanker), and set shifting (Set Shifting), and two verbally-mediated tests of generativity (D-word and Animal fluency). All computerized tasks included a minimum of 3 practice trials. This battery of executive functioning tasks is described in detail elsewhere and is a part of the NIH EXAMINER battery[39].

In brief, for *Dot Counting*, participants were instructed to count and recall the number of target dots in the order they were presented on a 2–6 series of screens. Visual *1-Back* and *2-Back* tasks asked participants to judge if a target square was in the same location as the preceding square (1-Back) or with the square two before it (2-Back). In *Enclosed Flanker,* participants were asked to push the key corresponding only to the direction of a target arrow, ignoring other distractor arrows. During *Set Shifting,* participants were asked to match a sample object to choice objects either by *shape* or by *color*. *Verbal generativity tasks* included lexical (number of D-words generated in 60”) and semantic (number of animals generated in 60”) fluencies.

Processing Speed—To assess processing speed, a composite score was derived from 5 computerized tests of *reaction time* to visual stimuli (Dots, Lines, Search, Shapes, Abstract Matching 1, Abstract Matching 2). All tasks included a practice trial period where the subject had to perform at > 70% accuracy to continue to the test trials, and have been validated as sensitive behavioral markers of brain aging[40].

During *Dots,* participants were instructed to select which of two dots was closest to a target central dot. *Lines* required participants to indicate which of two parallel lines was longer. For *Search*, participants were then asked whether a target was present in an array of shapes. During *Shapes*, participants were asked to judge which of the two choice shapes was most similar to a target shape. For *Abstract Matching 1*, participants were instructed to select which of two choice arrays was most similar to the sample array. *Abstract Matching 2* mirrored *Abstract Matching 1*, but added an additional level of complexity (orientation).

Geriatric Depression Scale (GDS)

Given that one of our primary variables of interest was perceived stress, and there is an important relationship between mood and self-reported scales, we adjusted for depressive symptoms in all relevant analyses to more directly measure the effects of stress on inflammation (versus general mood). The GDS is a 30-item self-report screener of depressive symptoms for older adult populations. Each question is scored 0 or 1 point, with a total of 0–10 considered normal/minimal, 11–20 moderate, and 21–30 severe depressive symptoms.

Statistical Analyses

Baseline univariable associations were examined with the primary variables of interest (age, monocyte/macrophage concentrations, and cognition) utilizing Spearman's rho correlations for nonparameteric data.

Next, unstructured mixed-effects linear regression models allowing for individual-specific intercepts and slopes were conducted to evaluate the relationships between baseline levels of perceived stress and age (time-varying) on monocyte/macrophage cytokine concentrations across time. Mixed-effect modeling was selected due to its robust ability to model repeated time points with varying intervals and visit numbers. Specifically, we specified random effects for person and time, and fixed effects included baseline perceived stress, age (time-varying), and their interaction (baseline stress*age), adjusting for sex, education, and baseline Geriatric Depression Scale (GDS) total score, predicting monocyte/macrophage cytokine levels across time. In this manner, the stress*age term assessed the effects of baseline stress dependent on age. Age was centered for all mixed-effects models (mean age = 73).

We then developed post-hoc models to probe the age*stress interaction term. Specifically, given our primary aim was to examine moderators of the relationship between aging and inflammation, we applied a median split to the Perceived Stress Scale (median = 9) to separate participants into baseline "high" (n=520) or "low" (n=553) stress groups. Additionally, given that the omnibus model included age as a time-varying parameter, we aimed to disentangle the biological effects of age from the effects of study time on our outcome. Therefore, we entered age at baseline (time invariant) and study time (years) as separate parameters. Our final post-hoc models included baseline age, study time, their interaction (baseline age*time), and covariates (sex, education, baseline GDS), predicting monocyte/macrophage cytokine levels. Therefore, the main effect of age represented the biological impact of age on the outcomes, time represented how monocyte/macrophage

cytokine levels changed with time in study, and their interaction indicated how age impacted the trajectories of monocyte/macrophage cytokines across time.

To support the clinical relevance of the monocyte/macrophage models, we examined the relationship between monocyte/macrophage cytokines (time-varying) and cognition (time-varying) across time (adjusting for baseline age, education, and sex) again via linear mixed-effects modeling. Additionally, we aimed to test our full "double hit" hypothesis that stress would potentiate age effects on inflammation, which would have a downstream negative impact on cognition. Therefore, based on our post-hoc monocyte/macrophage models, we developed parallel mediation models in the "high" and "low" stress groups (Perceived Stress Scale median split = 9). Specifically, we examined the degree to which time-varying monocyte/macrophage cytokines mediated the relationship between baseline age and time-varying cognition in individuals reporting high or low baseline stress separately, adjusting for sex, education, and baseline GDS. First, we estimated the Total Effect of baseline age on time-varying cognition, adjusting for time, baseline age*time, sex, education, and baseline GDS in each stress group. Second, we estimated the Direct Effect of baseline age on time-varying cognition, adjusting for time-varying monocyte/macrophage cytokines, as well as time, baseline age*time, sex, education, and baseline GDS in each stress group. Finally, we estimated the indirect effect by assessing the proportion of the Total Effect of baseline age on cognition that was explained when monocyte/macrophage cytokines was entered into the model: 1 − [(direct effect) / (total effect)]. Bootstrapping package in STATA was used to generate a bootstrap distribution for the percentage of attenuation (i.e., indirect effect) in each stress group based on 1000 resamples to calculate a 95% confidence interval for the indirect effect.

RESULTS

At baseline, older age was associated with higher monocyte/macrophage cytokine concentrations (Spearman's rho=0.43, n=298, p<0.001) and poorer cognitive performances (CVLT-II delayed recall Spearman's rho=−0.23, n=355, p<0.001; executive functions Spearman's rho=−0.29, n=366, p<0.001; processing speed Spearman's rho=0.30, n=260, p<0.001), as expected, but was not significantly associated with perceived stress (Spearman's rho=0.03, n=298, p=0.60). Greater perceived stress was associated with slower processing speed (Spearman's rho=0.19, n=260, p=0.02), but was otherwise not significantly associated with the other cognitive measures (CVLT-II delayed recall Spearman's rho=0.01, n=355, p=0.83; executive Spearman's rho=−0.08, n=366, p=0.11) or the macrophage composite (Spearman's rho=0.07, n=298, p=0.23).

Longitudinal mixed-effects models demonstrated a significant age (time-varying) by baseline stress interaction predicting changes in monocyte/macrophage cytokine levels (baseline stress*age b=−0.01, t(404)=2.2, p=0.03; see Table 2). To probe the interaction, we applied a median split to baseline Perceived Stress Scale scores (median=9) and conducted parallel mixed-effects models in "high" and "low" stress groups, separately. Additionally, to disentangle the biological effect of age from the effect of time, we created a baseline age parameter (time invariant) and a study time (years) parameter. The final post-hoc models included baseline age, study time, their interaction (baseline age*time), and covariates of

interest (sex, education, baseline GDS) predicting monocyte/macrophage concentrations. In low stress individuals, there was only a negative main effect of baseline age on macrophage levels (Model AIC=743.1, BIC=777.9; baseline age b=0.44, t(236)=7.7, p<0.001; time b= −0.006, t(308)=−0.42, p=0.68; baseline age*time=−0.005, t(310)= −0.32, p=0.75). However, in high stress individuals, there was a significant baseline age by time interaction (Model AIC=658.6, BIC=693.0; baseline age b=0.20, t(183)=3.7, p<0.001; time b=0.02, t(248)=1.1, p=0.26; baseline age*time b=0.03, t(248)=2.1, p=0.039; Table 2). Specifically, in high stress, older baseline age was associated with disproportionately steeper elevations in macrophage levels across time (Figure 2). Interpreting the effect sizes, at the average baseline age (73 years), individuals reporting high stress were estimated to demonstrate a 0.02 z-score *increase* in macrophage cytokines each year, compared to a 0.006 z-score *decrease* in macrophage cytokines in the low stress individuals. Importantly, the interaction term then indicated that with each 1-year increase of age, there was a 2-fold elevation in macrophage cytokine trajectories over time in the high stress adults. Directly comparing the interaction parameters in the high versus low stress models, there was a 5.8-fold increased elevation in macrophage cytokine trajectories per year in the high compared to low stress adults.

To clinically anchor the inflammatory biomarker results, longitudinal mixed-effects models examining the relationship between changes in cytokines and changes in cognition were conducted and demonstrated that increases in monocyte/macrophage cytokine levels were associated with declines in executive functioning performances across time (Model *AIC*=1074.5, *BIC*=1106.4; macrophage composite *b*=−0.07, t(718)= −2.44, *p*=0.02). The relationship between changes in monocyte/macrophage cytokines and changes in CVLT-II delayed recall or processing speed did not reach significance (CVLT-II: Model *AIC*=3243.2, *BIC*=3274.9; macrophage composite *b*=−0.24, t(662)=−1.64, *p*=0.10; Processing Speed: Model *AIC*=1361.5, *BIC*=1389.8; macrophage composite *b*=−0.11, t(371)=−1.29, *p*=0.20).

Finally, we developed parallel latent growth models (bootstrapped with 1,000 replications) examining the mediating effect of monocyte/macrophage cytokines on the relationship between age and executive functions in the high stress (PSS>9) and low stress (PSS≤9), separately. Only the mediation model in the high stress group was significant, demonstrating that 19.1% of relationship of age on executive functions was mediated by age-related changes in monocyte/macrophage levels (z=2.09, n=144, bootstrap p=0.036, 95%CI 0.01, 0.37), compared to 6.9% in the low stress group (z=0.91, n=151, bootstrap p=0.91, 95%CI −0.08, 0.22) (see Figure 3).

CONCLUSIONS

Higher perceived stress at baseline was associated with disproportionately steeper elevations in monocyte/macrophage cytokine concentrations than expected for one’s age across time in otherwise clinically normal aging adults. Additionally, not only did increases in monocyte/macrophage cytokines track with decreases in executive functions over time, but monocyte/macrophage cytokines partially mediated the relationship between age and executive functions *only in individuals reporting high perceived stress* at baseline. Together, these results support a pattern of “accelerated aging” of the immune systems in adults with higher stress levels that may have measureable clinical ramifications on executive functioning

performances. That is, the relationships among age, monocyte/macrophage inflammation, and executive functions were more tightly coupled within the high stress group. Importantly, all models were significant independent of mood (Geriatric Depression Scale), suggesting some specificity of these effects to stress versus general affective state. Perceived stress clearly impacts neurobehavior in aging[1–5], and our results suggest that this may at least be in part associated with monocyte/macrophage-related inflammatory activation.

In aging, macrophage homeostasis shifts from a state of quiescence to primed activation, ready to react to perceived threats or injury[7]. Indeed, we found overall higher concentrations of monocyte/macrophage cytokines in older ages, and that stress appeared to be an immune potentiator such that in high stress individuals there were even higher trajectories of inflammatory cytokines across time than their same aged peers. Of course, the directionality of these results warrants consideration. While we might hypothesize that stress triggers an immune response, it is equally possible given the multiple feedback loops of the immune system, that greater macrophage inflammation could instigate or further exacerbate perceived stress[18]. Our data are among the first to probe markers outside of IL-6 or TNF-α (considered more non-specific master regulators) in the context of clinical stress. Inclusion of MIP1-α and MIP1-β proteins may be more monocyte/macrophage-specific, supporting its potential role in the human aging stress response. However, it is important to note that while our *a priori* hypotheses and study model were developed around the role of monocyte/macrophage activation, the cytokine markers we measured are not only specific to macrophage functioning and we may, in fact, be capturing other important pathways in the heterogeneous picture of aging immunosenescence. Though all cytokine markers measured are produced by macrophages and further feedback to potentiate macrophage activation, each then also instigates activation of a host of other cell types not specific to monocytes (e.g., neutrophils, NK cells) both within the innate and also adaptive immune system[31,36,37]. Additionally, age-related deficits in other non-monocyte cells, such as lymphocytes (e.g., NK cells), directly communicate with monocytes/macrophages resulting in alterations in cytokine release[20,23,24]. Therefore, it is possible that our results may be reflecting the effects of stress on age-related changes in immunosenescence more broadly. Nonetheless, ours are among the first data to support an age-related accelerated immune activation related to stress in humans, and begins to translate important inflammatory-stress animal models into the clinical realm.

We also found that greater monocyte/macrophage activation tracked with worse executive functioning performances across time, and that monocyte/macrophage activation partially mediated the relationship between age and executive functions only within adults reporting high stress levels. These findings begin to support that concept the potentiating effects of stress on age-related inflammation trajectories may have important clinical correlates in humans. Specifically, among individuals with higher levels of baseline perceived stress, the relationships among age, monocyte/macrophage inflammation, and executive functions appeared more tightly intertwined -- 19% of the relationship between age and executive functions was explained through our inflammatory markers. On the other hand, in low stress individuals, the mediation model was not significant and only ~6% of the relationship between age and executive functions appeared to be mediated by the inflammatory markers. While macrophages are first-line immune proteins found throughout the body and we

measured inflammation peripherally, given their ability to signal across the blood-brain barrier, clear expression in the CNS[41], and our demonstrated relationship with cognition, it is tempting to extrapolate the importance of our findings to brain health. Within the CNS, microglia are the resident macrophages that support phagocytosis of unwanted cell debris[22]. While their role actively surveying synapses and dynamically reacting to neural transmission facilitates developmental synaptic pruning, recent theories have begun to posit that dysfunction of these glial processes in older age may result in aberrant pruning and possibly, trigger neurodegeneration[10,42]. In the context of our findings, stress may increase already heightened microglial reactivity, resulting in overactive phagocytosis that may be come cytotoxic (e.g., indiscriminate “pruning”), and contribute to poorer CNS functioning.

These data are also commensurate with ongoing literature demonstrating the detrimental role of stress on accelerated prefrontal atrophy and executive dysfunction in humans[3,6]. Prefrontal regions are also the neural networks that undergo the most significant changes in aging, including reduced synaptic density, desynchronized neural activation, and overt atrophy, and are vulnerable to Alzheimer’s pathology aggregation. Taken together, stress may play a potential moderating role in less optimal and even pathologic brain aging trajectories and therefore be a largely modifiable and high impact intervention target. Initiation of empirically-based behavioral therapies for stress, such as Mindfulness-Based Stress Reduction[43,44], appear highly warranted in individuals experiencing stress in aging which may directly moderate inflammation trajectories, and downstream, benefit cognition.

These data are not without limitations. Namely, while our primary question of interest centered on age-related CNS immune and macrophage functioning, we measured inflammatory cytokines peripherally and these cytokines are involved in multiple pathways involving but also extending beyond macrophages. Notably, the measured cytokines reliably demonstrate the ability to cross and signal across the blood-brain barrier[28–30], and our recent work suggests that at least one of our more macrophage-specific markers, MIP1-β, is significantly correlated in plasma and cerebrospinal fluid (r=0.55)[41]. Additionally, we demonstrated that our macrophage cytokine composite was associated with clinically relevant CNS functioning (i.e., cognition). Nonetheless, the global, non-specific effects of systemic inflammation/immunosenescence may likely also be contributing to and playing a role in the observed effects. In addition, though ours is among the first studies to specifically examine stress in the context of macrophage-related cytokines in humans and did so longitudinally, our longitudinal data are limited by the number of observations. Given that the median number of visits was 2 and only 46% had >2 time-points, our ability to detect change may have been limited by power and we were less able to model other nonlinear trajectories across time. Lastly, though our perceived stress scale has been widely validated and used to examine the effects of age-related stress previously[17], it is subjective (vs. cortisol levels) and intended to measure negative and “out of control” stress. While stress that is perceived to be out of one’s control is consistently associated with worse CNS outcomes, low levels of (self-perceived) controlled stress may actually be beneficial for challenging the immune system and brain[45,46]. Future works that appreciate the full spectrum of stress, both positive and negative, on the aging brain are highly warranted to optimally shape treatment approaches and clinical recommendations.

In sum, higher perceived stress appears to accelerate the detrimental effects of aging on macrophage-related cytokine trajectories, supporting the “double hit” hypothesis. These inflammatory trajectories also importantly mediate the relationship between age and executive functions in high, but not low stress individuals. These data begin to support that this effect may be related to monocyte/macrophage pathways in humans, and critically underscore the importance of stress-based interventions even in clinically normal aging. Mindfulness-Based Stress Reduction and other stress-related therapies (e.g., physical exercise) are highly warranted as primary and secondary prevention interventions in brain aging. Continued identification of the biological targets underlying behavioral aging phenomena have the potential to better refine and enhance the neural potency of intervention strategies to support the growing aging population and prevent neurodegeneration.

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References

1. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci. 2009; 10(6):434–445. DOI: 10.1038/nrn2639 [PubMed: 19401723]
2. Marin M-F, Lord C, Andrews J, et al. Chronic stress, cognitive functioning and mental health. 2011; doi: 10.1016/j.nlm.2011.02.016
3. Lupien S, Lecours A, Schwartz G, et al. Longitudinal study of basal cortisol levels in healthy elderly subjects: Evidence for subgroups. Neurobiol Aging. 1996; 17(1):95–105. DOI: 10.1016/0197-4580(95)02005-5 [PubMed: 8786810]
4. Bloss EB, Janssen WG, Mcewen BS, Morrison JH. Interactive Effects of Stress and Aging on Structural Plasticity in the Prefrontal Cortex. J Neurosci. 2010; 30(19):6726–6731. DOI: 10.1523/JNEUROSCI.0759-10.2010 [PubMed: 20463234]
5. Karlamangla AS, Singer BH, Chodosh J, McEwen BS, Seeman J. Urinary cortisol excretion as a predictor of incident cognitive impairment. Neurobiol Aging. 2005; 26(SUPPL 1):S80–S84. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=psyc4&NEWS=N&AN=2005-16648-017%5Cnhttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed10&NEWS=N&AN=41739432.
6. Franz CE, O’Brien RC, Hauger RL, et al. Cross-sectional and 35-year longitudinal assessment of salivary cortisol and cognitive functioning: The Vietnam Era Twin Study of Aging. Psychoneuroendocrinology. 2011; doi: 10.1016/j.psyneuen.2011.01.002
7. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging: An Evolutionary Perspective on Immunosenescence. Annals. 2000; 908:244–254.
8. Gomez CR, Boehmer ED, Kovacs EJ. The aging innate immune system. Curr Opin Immunol. 2005; 17(5):457–462. DOI: 10.1016/j.coi.2005.07.013 [PubMed: 16084711]
9. Bettcher BM, Fitch R, Wynn MJ, et al. MCP-1 and eotaxin-1 selectively and negatively associate with memory in MCI and Alzheimer’s disease dementia phenotypes. Alzheimer’s Dement Diagnosis, Assess Dis Monit. 2016; 3:91–97. DOI: 10.1016/j.dadm.2016.05.004
10. Perry VH. Contribution of systemic inflammation to chronic neurodegeneration. Acta Neuropathol. 2010; 120(3):277–286. DOI: 10.1007/s00401-010-0722-x [PubMed: 20644946]
11. Bettcher B, Kramer J. Longitudinal Inflammation, Cognitive Decline, and Alzheimer’s Disease: A Mini-Review. 2014; doi: 10.1038/clpt.2014.147

12. Kunz-Ebrecht SR, Mohamed-Ali V, Feldman PJ, Kirschbaum C, Steptoe A. Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. Brain Behav Immun. 2003; doi: 10.1016/S0889-1591(03)00029-1

13. Dhabhar FS, McEwen BS. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. Brain Behav Immun. 1997; 11(4):286–306. DOI: 10.1006/brbi.1997.0508 [PubMed: 9512816]

14. Perry VH, Newman TA, Cunningham C. The impact of systemic infection on the progression of neurodegenerative disease. Nat Rev Neurosci. 2003; 4(2):103–112. DOI: 10.1038/nrn1032 [PubMed: 12563281]

15. Glaser R, Kiecolt-Glaser JK, Marucha PT, Maccallum RC, Laskowski BF, Malarkey WB. Stress-Related Changes in Proinflammatory Cytokine Production in Wounds. Arch Gen Psychiatry. 1999; 56(5):450–456. [PubMed: 10232300]

16. Kiecolt-Glaser JK, Marucha PT, Mercado AM, Malarkey WB, Glaser R. Slowing of wound healing by psychological stress. Lancet. 1995; 346(8984):1194–1196. DOI: 10.1016/S0140-6736(95)92899-5 [PubMed: 7475659]

17. Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, Glaser R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. Proc Natl Acad Sci. 2003; doi: 10.1073/pnas.1531903100

18. Huang Y, Henry CJ, Dantzer R, Johnson RW, Godbout JP. Exaggerated sickness behavior and brain proinflammatory cytokine expression in aged mice in response to intracerebroventricular lipopolysaccharide. Neurobiol Aging. 2008; 29(11):1744–1753. DOI: 10.1016/j.neurobiolaging.2007.04.012 [PubMed: 17543422]

19. Sparkman NL, Johnson RW. Neuroinflammation associated with aging sensitizes the brain to the effects of infection or stress. Neuroimmunomodulation. 2008; doi: 10.1159/000156474

20. Solana R, Tarazona R, Gayoso I, Lesur O, Dupuis G, Fulop T. Innate immunosenescence: Effect of aging on cells and receptors of the innate immune system in humans. Semin Immunol. 2012; 24(5):331–341. DOI: 10.1016/J.SMIM.2012.04.008 [PubMed: 22560929]

21. Hanisch UK. Microglia as a source and target of cytokines. Glia. 2002; doi: 10.1002/glia.10161

22. Schafer DP, Lehrman EK, Stevens B. The “quad-partite” synapse: Microglia-synapse interactions in the developing and mature CNS. Glia. 2013; 61(1):24–36. DOI: 10.1002/glia.22389 [PubMed: 22829357]

23. Montgomery RR, Shaw AC. Paradoxical changes in innate immunity in aging: recent progress and new directions. J Leukoc Biol. 2015; 98(6):937–943. DOI: 10.1189/jlb.5MR0315-104R [PubMed: 26188078]

24. Metcalf TU, Wilkinson PA, Cameron MJ, et al. Human Monocyte Subsets Are Transcriptionally and Functionally Altered in Aging in Response to Pattern Recognition Receptor Agonists. J Immunol. 2017; 199(4):1405–1417. DOI: 10.4049/jimmunol.1700148 [PubMed: 28696254]

25. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer’s disease: Recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimer’s Dement. 2011; 7(3):263–269. DOI: 10.1016/j.jalz.2011.03.005 [PubMed: 21514250]

26. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav. 1983; 24(4):385–396. DOI: 10.2307/2136404 [PubMed: 6668417]

27. Cohen S, Williamson G. Perceived stress in a probability sample of the United States. Soc Psychol Heal. 1988; 13:31–67. DOI: 10.1111/j.1559-1816.1983.tb02325.x

28. Czaplewski LG, McKeating J, Craven CJ, et al. Identification of amino acid residues critical for aggregation of human CC chemokines macrophage inflammatory protein (MIP)-1alpha, MIP-1beta, and RANTES. Characterization of active disaggregated chemokine variants. J Biol Chem. 1999; 274(23):16077–16084. DOI: 10.1074/jbc.274.23.16077 [PubMed: 10347159]

29. Fahey TJ, Tracey KJ, Tekamp-Olson P, et al. Macrophage inflammatory protein 1 modulates macrophage function. J Immunol. 1992; 148(9):2764–2769. [PubMed: 1573267]

30. Passos GF, Figueiredo CP, Prediger RDS, et al. Role of the macrophage inflammatory protein-1alpha/CC chemokine receptor 5 signaling pathway in the neuroinflammatory response

and cognitive deficits induced by beta-amyloid peptide. Am J Pathol. 2009; 175(4):1586–1597. DOI: 10.2353/ajpath.2009.081113 [PubMed: 19729478]

31. Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. Cytokine Growth Factor Rev. 2002; 13(6):455–481. [Accessed February 2, 2018] http://www.ncbi.nlm.nih.gov/pubmed/12401480. [PubMed: 12401480]
32. Schall TJ, Bacon K, Camp RD, Kaspari JW, Goeddel DV. Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. J Exp Med. 1993; 177(6):1821–1826. DOI: 10.1084/jem.177.6.1821 [PubMed: 7684437]
33. Tessier PaNaccache PH, Clark-Lewis I, Gladue RP, Neote KS, McColl SR. Chemokine networks in vivo: involvement of C-X-C and C-C chemokines in neutrophil extravasation in vivo in response to TNF-alpha. J Immunol. 1997; 159(7):3595–3602. [PubMed: 9317159]
34. Smith RE, Strieter RM, Phan SH, Lukacs N, Kunkel SL. TNF and IL-6 mediate MIP-1alpha expression in bleomycin-induced lung injury. J Leukoc Biol. 1998; 64(4):528–536. http://www.ncbi.nlm.nih.gov/pubmed/9766634. [PubMed: 9766634]
35. Coppack SW. Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc. 2001; 60(3):349–356. [Accessed February 2, 2018] http://www.ncbi.nlm.nih.gov/pubmed/11681809. [PubMed: 11681809]
36. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014; 6(10):a016295.doi: 10.1101/cshperspect.a016295 [PubMed: 25190079]
37. Idriss HT, Naismith JH. TNFα and the TNF receptor superfamily: Structure-function relationship(s). Microsc Res Tech. 2000; 50(3):184–195. DOI: 10.1002/1097-0029(20000801)50:3<184::AID-JEMT2>3.0.CO;2-H [PubMed: 10891884]
38. Delis DC, Kramer JH, Kaplan E, Ober BA. California Verbal Learning Test – second edition. Adult version. Manual. Test. 2000
39. Kramer JH, Mungas D, Possin KL, et al. NIH EXAMINER: Conceptualization and Development of an Executive Function Battery. J Int Neuropsychol Soc. 2014; 20(1):11–19. DOI: 10.1017/S1355617713001094 [PubMed: 24103232]
40. Kerchner GA, Racine CA, Hale S, et al. Cognitive Processing Speed in Older Adults: Relationship with White Matter Integrity. PLoS One. 2012; 7(11)doi: 10.1371/journal.pone.0050425
41. Bettcher BM, Johnson SC, Fitch R, et al. CSF and Plasma Levels of Inflammation Differentially Relate to CNS Markers of Alzheimer's Disease Pathology and Neuronal Damage. Alzheimer's Dement.
42. Riccomagno MM, Kolodkin AL. Sculpting Neural Circuits by Axon and Dendrite Pruning. Annu Rev Cell Dev Biol. 2015; 31(1):779–805. DOI: 10.1146/annurev-cellbio-100913-013038 [PubMed: 26436703]
43. Kabat-Zinn J. Mindfulness-based interventions in context: Past, present, and future. Clin Psychol Sci Pract. 2003; 10(2):144–156. DOI: 10.1093/clipsy/bpg016
44. Baer RA, Carmody J, Hunsinger M. Weekly Change in Mindfulness and Perceived Stress in a Mindfulness-Based Stress Reduction Program. J Clin Psychol. 2012; 68(7):755–765. DOI: 10.1002/jclp.21865 [PubMed: 22623334]
45. Hadad-Ophir O, Ardi Z, Brande-Eilat N, Kehat O, Anunu R, Richter-Levin G. Exposure to prolonged controllable or uncontrollable stress affects GABAergic function in sub-regions of the hippocampus and the amygdala. Neurobiol Learn Mem. 2017; 138:271–280. DOI: 10.1016/j.nlm.2016.06.009 [PubMed: 27321164]
46. Varela JA, Wang J, Christianson JP, Maier SF, Cooper DC. Control over Stress, But Not Stress Per Se Increases Prefrontal Cortical Pyramidal Neuron Excitability. J Neurosci. 2012; 32(37):12848–12853. DOI: 10.1523/JNEUROSCI.2669-12.2012 [PubMed: 22973008]

Highlights

- Stress and aging are associated with macrophage and microglia activation in animals.
- High stress adults demonstrated accelerated age-related macrophage cytokine elevations.
- Macrophage cytokines mediated the relationship between age and cognition in high stress adults.
- Stress interventions may target age-related inflammation to benefit cognition in aging.

Significance

We demonstrate accelerated elevations in age-related macrophage cytokine in otherwise healthy adults, and that these elevations mediate changes in age-related executive functions across time. Our data are the first to support a potential macrophage-specific role to the relationship between stress and cognitive aging in humans. Macrophage activation is importantly implicated in aberrant synaptic pruning and neurodegeneration. These findings support early stress-based therapies to alter immune and cognitive aging trajectories.

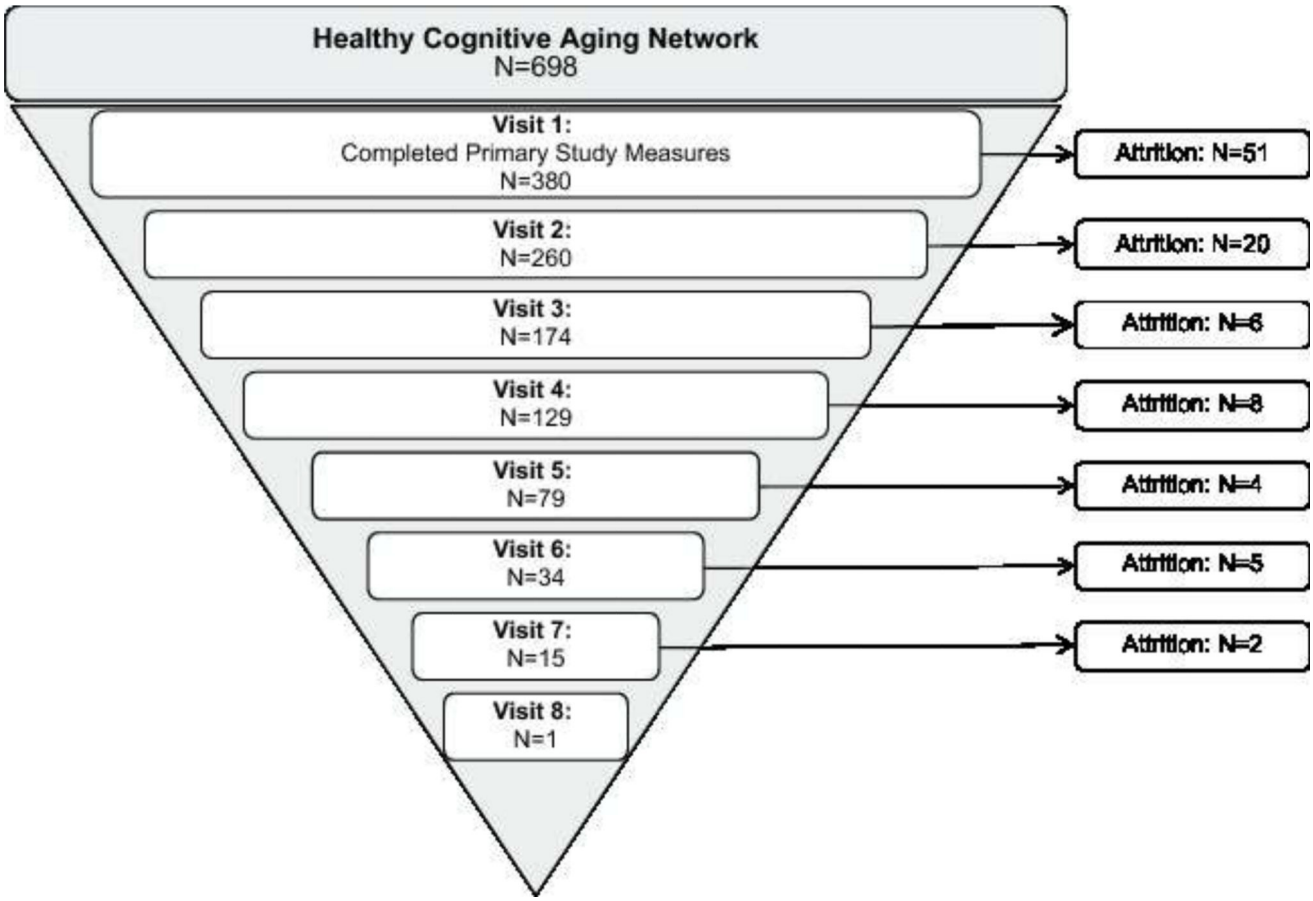

Figure 1.
Older adult participants included in the current study pulled from the larger UCSF Healthy Cognitive Aging Network.

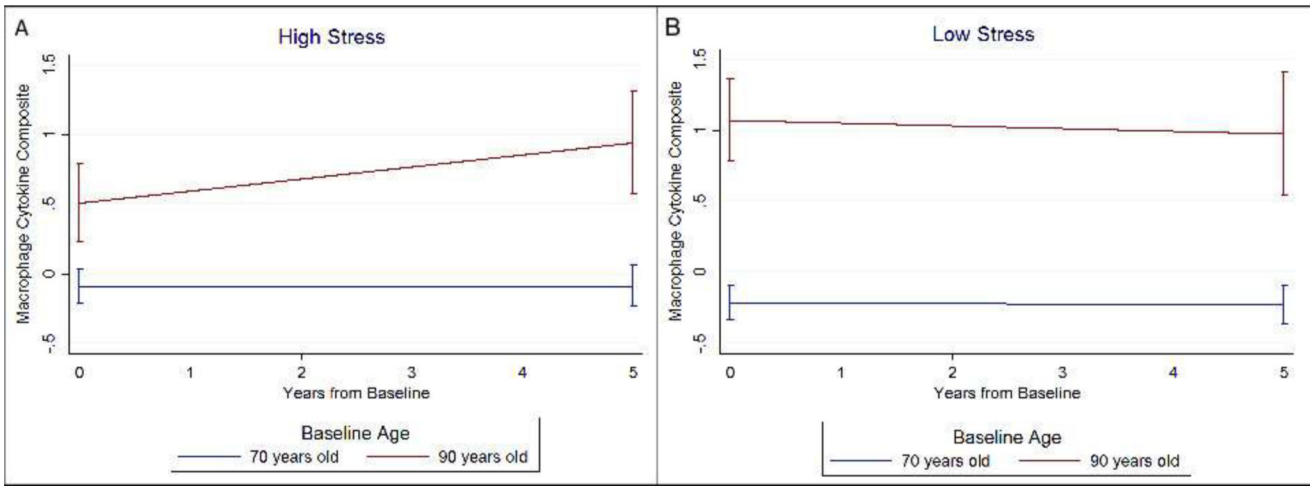

Figure 2.

Baseline age interacts with time to predict accelerated monocyte/macrophage cytokine trajectories only among adults with high (panel A) but not low (panel B) baseline perceived stress. Error bars represent 95% confidence intervals.

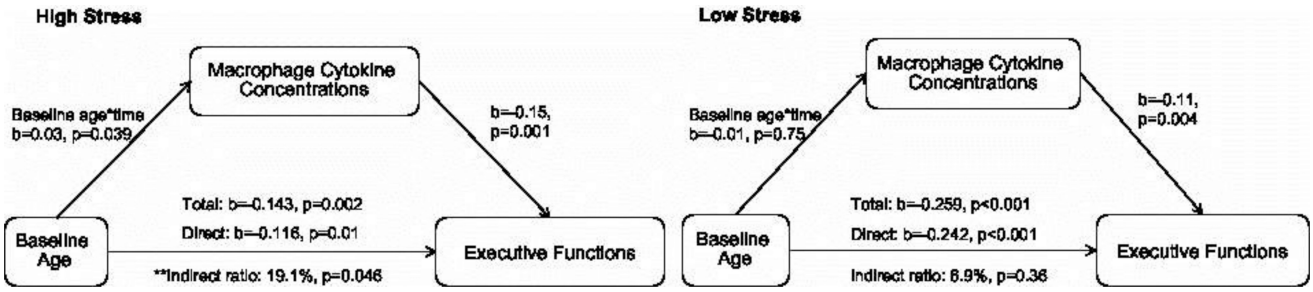

Figure 3.

Monocyte/macrophage cytokine levels significantly mediate the relationship between age and executive functions only among adults with high, but not low baseline perceived stress.

Note. Test statistics reflect latent growth curve bootstrapped mediation models (1,000 replications) adjusted for time, sex, education, and GDS at baseline. b = slope coefficient and p = bootstrapped p-value. High Stress model n=144 with 329 observations; Low Stress model n=151 with 352 observations.

Table 1

Baseline study participant demographic and clinical characterization.

	***Mean* (*SD*) or *Median* (*IQR*)**
Study Visits	2.5 (1.5) (range: 1, 8)
Cumulative Years Followed	2.8 (2.5) (range: 0, 7.8)
Age, years	73.3 (6.8)
Sex, % female	55.6%
Education, years	17.5 (2.1)
Perceived Stress Scale, total score	9.5 (5.4) (range: 0, 26)
Plasma Inflammation Markers (pg/mL)	
IL-6	0.83 (0.69)
TNF-α	2.6 (0.85)
MIP1-α	14.7 (7.0)
MIP1-β	61.9 (29.9)
Geriatric Depression Scale	2 (0, 4)
Baseline Cognition	
MMSE	29 (29, 30)
CVLT-II Long delay free recall, raw score	11.7 (3.1)
Executive composite, z-score	0.8 (0.6)
Processing Speed composite, z-score	2.4 (1.3)

Note. MMSE = Mini-Mental State Examination; CVLT-II = California Verbal Learning Test, second edition.

Table 2

Omnibus and post-hoc longitudinal models demonstrating significant baseline stress effects on aging-related monocyte/macrophage levels.

	BIC	beta	t ratio (df)	95% CI	p-value
DV: Monocyte/Macrophage Cytokines					
Model	1453.1				<0.001
Sex		0.01	0.19 (283)	−0.06, 0.08	0.85
Education		−0.02	−0.93 (271)	−0.05, 0.02	0.35
GDS (baseline)		−0.01	−1.19 (264)	−0.04, 0.01	0.24
Age		0.40	5.96 (273)	0.27, 0.54	<0.001
Baseline Stress		0.01	1.68 (272)	−0.002, 0.03	0.09
Age*Baseline Stress		***−0.01***	−2.15 (404)	***−0.02, −0.001***	***0.03***
High Baseline Stress					
DV: Monocyte/Macrophage Cytokines					
Model	693.0				<0.001
Sex		−0.001	−0.02 (183)	−0.11, 0.11	0.98
Education		−0.02	−0.75 (124)	−0.07, 0.03	0.45
GDS (baseline)		−0.02	−1.05 (126)	−0.04, 0.01	0.29
Age (baseline)		0.20	3.69 (183)	0.09, 0.31	0.003
Study time		0.015	1.13 (248)	−0.01, 0.04	0.26
Age (baseline)*Time		***0.029***	**2.07 (249)**	***0.001, 0.06***	***0.039***
Low Baseline Stress					
DV: Monocyte/Macrophage Cytokines					
Model	777.9				<0.001
Sex		0.005	0.09 (158)	−0.09, 0.10	0.93
Education		−0.006	−0.23 (151)	−0.05, 0.04	0.82
GDS (baseline)		−0.009	−0.43 (150)	−0.05, 0.03	0.67
Age (baseline)		0.44	7.72 (236)	0.33, 0.55	<0.001

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	BIC	beta	t ratio (df)	95% CI	p-value
Study time		−0.006	−0.42 (308)	−0.04, 0.02	0.68
Age (baseline)*Time		−0.005	−0.32 (310)	−0.04, 0.03	0.75

Note. Age is centered; Baseline Stress = Perceived Stress Scale (PSS, range: 0–26); High Baseline Stress = PSS > 9, Low Baseline Stress = PSS ≤ 9.